2008

Population Growth and Development of Psocid *Lepinotus reticulatus* at Constant Temperatures and Relative Humidities

G. P. Opit
USDA-ARS, george.opit@ars.usda.gov

James E. Throne
USDA-ARS, Manhattan, KS, james.throne@ars.usda.gov

Population Growth and Development of Psocid *Lepinotus reticulatus* at Constant Temperatures and Relative Humidities

G. P. OPIT[1] AND J. E. THRONE

USDA–ARS, Grain Marketing and Production Research Center, 1515 College Avenue, Manhattan, KS, 66502-2736

J. Econ. Entomol. 101(2): 605–615 (2008)

ABSTRACT We investigated the effects of temperature and relative humidity on population growth and development of the psocid *Lepinotus reticulatus* Enderlein. Part of this study assessed the effects of marking psocids by using methylene blue, chalk powder, and fluorescent powder to differentiate nymphal stages during development. We found that marking psocids by using methylene blue increased mortality and took twice as long to accomplish compared with marking by using fluorescent powder. Using chalk powder shortened the duration of third and fourth nymphal instars. Marking psocids by using fluorescent powder had no effect on mortality or duration of nymphal instars. Therefore, we recommend using fluorescent powder for marking psocids. *L. reticulatus* did not survive at 32, 43, and 55% RH, whereas populations increased from 22.5 to 32.5°C at 75% RH. The largest population growth was recorded at 30 and 32.5°C, whereas only 9% of nymphs developed to adults and populations declined at 35°C. We developed temperature-dependent developmental equations for eggs, individual nymphal, combined nymphal, and combined immature stages. These equations showed predicted optimal temperatures for the development of eggs, combined nymphal, and combined immature stages to be 32.3, 34.5, and 34.4°C, respectively; development at these temperatures was completed in 6.3, 16.7, and 23.3 d, respectively. Our study shows that psocids that consume their exuviae develop faster than those that do not, and this effect is more pronounced at lower temperatures. These data give us better understanding of *L. reticulatus* population dynamics, and they can be used to develop effective management strategies for this psocid.

KEY WORDS psocids, marking, stored products, developmental rates, life history

Psocids are an emerging problem in grain storages, grain processing facilities, and product warehouses in the United States and many other countries. The rise of psocids to prominence can be attributed to their varied response to management tactics that have been developed for beetle pests, e.g., some psocid species are resistant to residual insecticides and the fumigant phosphine, whereas others are not (Nayak et al. 1998, 2002a, 2002b, 2003; Nayak 2006). In addition, markets increasingly view psocids as contaminants (Nayak 2006).

We conducted studies in 2004 to determine which species of psocids were present in wheat stored in steel bins in Manhattan, KS (Throne et al. 2006), and we found only *Liposcelis entomophila* (Enderlein) (Psocoptera: Liposcelididae) and *Lepinotus reticulatus* Enderlein (Psocoptera: Trogiidae). Both species are cosmopolitan (Lienhard and Smithers 2002). There have been a few studies on biology of *L. entomophila* (Leong and Ho 1995; Wang et al. 1998; Mashaya 1999, 2001). However, we are aware of only one brief study on the biology of a member of the genus *Lepinotus*, *L. patruelis* (Finlayson 1949). *L. reticulatus* is an obligate parthenogen (Mockford 1993). The only ecological research conducted on *L. reticulatus* was an investigation of its population dynamics in farm-stored grain (Sinha 1988). Development of an effective pest management program is dependent on having sound knowledge of pest ecology. Given the limited information on ecology of *L. reticulatus*, we initiated studies on population growth and development of *L. reticulatus* to provide an experimental basis for developing pest management strategies for this pest.

Published studies on stage-specific development rates of psocids have used methylene blue to differentiate developmental stages (Mockford 1957, Leong and Ho 1995). This is because the different stages are not readily identified using morphological characters (Finlayson 1949) and because timing of psocid molts cannot be reliably determined by observing cast skins due to the tendency of psocids to consume their exuviae. However, marking psocids by using methylene blue is time-consuming and requires finesse to avoid mechanical injury (Leong and Ho 1995), death due to drowning in methylene blue, or getting crushed by the

Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

[1] Corresponding author, e-mail: george.opit@ars.usda.gov.

applicator. Unhealthy or injured insects may provide spurious life cycle data, compromising the predictive ability of models developed using such data (Leong and Ho 1995). Using chalk powder or fluorescent powder to mark insects is possible alternative to methylene blue. Psocids have hairy abdomens on which colored particles of chalk powder and fluorescent powder can firmly attach, facilitating the marking process. If chalk and fluorescent powder have no unintended effects on psocids, they could be used as a replacement for methylene blue as a means of marking psocids.

Our objectives were to determine the effects of constant temperature and relative humidity on population growth of *L. reticulatus*, to assess the effects on psocids of marking using chalk powder and fluorescent powder, and to quantify the effects of temperature on development of *L. reticulatus*. This information will be useful in developing management strategies for this pest.

Materials and Methods

Insects. Cultures used in the study were started with insects collected during summer 2004 in wheat, *Triticum aestivum* L., stored in steel bins at the Grain Marketing and Production Research Center in Manhattan, KS. Voucher specimens of *L. reticulatus* used in this study were deposited in the Kansas State University Museum of Entomological and Prairie Arthropod Research under Lot Number 181. Psocids were reared on a mixture of 97% cracked hard red winter wheat, 2% rice krispies, and 1% brewer's yeast (wt:wt; referred to as psocid diet below) in 0.473-liter glass canning jars covered with mite-proof lids. Cultures were maintained at 30°C and 75% RH.

The mite-proof lid prevents mites from entering the jar, while permitting air movement. The mite-proof lid is made by punching a 4-cm-diameter hole in the 6-cm-diameter canning jar lid. The hole is then covered by soldering a 5-cm-diameter U.S. #40 mesh (0.42-mm openings) brass screen to the outside of the lid. A 7-cm-diameter filter paper is then placed on either side of the lid, and the lid is placed in the band used to secure the lid to the jar.

Effects of Temperature and Relative Humidity on Population Growth. We determined effects of temperature and relative humidity on increase in number of psocids over a 46-d period at six temperatures (22.5, 25.0, 27.5, 30.0, 32.5, and 35.0°C) and four relative humidities (32, 43, 55, and 75%). The top third of the inner surface of 144 vials was coated with Fluon (polytetrafluoroethylene; Northern Products, Woonsocket, RI) to prevent psocids from escaping, and 5 g of psocid diet was placed in each vial. A screen (U.S. #40 mesh) was placed in the snap-cap lid to allow air movement. Vials were randomly placed in each of four plastic boxes (40 by 27.5 by 16 cm in height) containing saturated solutions of $MgCl_2$, K_2CO_3, NaBr, and NaCl below perforated false floors to maintain relative humidities of 32, 43, 55, and 75% (Greenspan 1977), respectively, and the diet in the vials was equilibrated at room temperature for 4 wk.

One- to 2-wk-old female *L. reticulatus* for the experiment were obtained by placing 1 g of colored psocid diet, 10 particles of cracked wheat, and 30 adult female psocids of unknown age from our culture in each of eighty 35-mm-diameter petri dishes (Greiner Bio-One, Kaysville, UT), which had a coat of Fluon on the walls to prevent psocids from escaping. Colored diet was made by mixing 100 g of rice krispies with a solution of 5 ml of red food dye (Global ChemSources Inc., Cedar Grove, NJ) in 300 ml of water, drying the mixture in a mechanical convection oven (model HTM 85, Precision Scientific, Inc., Chicago, IL) over a 2-d period, and then grinding the dried mixture in a Wiley Mill by using a #20 sieve (0.85-mm openings) (Scientific Apparatus, Philadelphia, PA). Colored diet was used because *L. reticulatus* prefers laying eggs between diet particles, and colored diet makes it easier to see eggs and, therefore, to make an assessment of whether sufficient numbers of eggs are being laid for the experiment. The petri dishes were placed on false floors in three Rubbermaid plastic boxes (30 by 23 by 9 cm in height) that contained saturated NaCl solution. The boxes had been painted black to exclude light and mimic dark conditions inside steel grain bins where the *L. reticulatus* used to start our culture were obtained. Boxes were placed in an incubator maintained at 30 ± 1°C and 70 ± 5% RH. Psocids were removed from each petri dish after 7 d, and the contents of all the petri dishes were poured into an 800-ml glass jar containing 250 g of psocid diet. The top part ("neck") of the jar had a coat of Fluon and was closed using a mite-proof lid. The jar was placed back in the incubator. After 30 d, adult psocids found in the jar were ≈1–2 wk old (based on preliminary work we had done which indicated that development from egg to adult took ≈25 d at 30°C).

Five 1- to 2-wk-old adult females were added to each of the 144 vials containing equilibrated diet, which were then incubated at each of the 24 temperature–relative humidity combinations. Six incubators were set at temperatures of 22.5, 25.0, 27.5, 30.0, 32.5, and 35.0°C, and into each incubator were placed four plastic boxes (20 by 12.5 by 10 cm in height) containing saturated solutions of $MgCl_2$, K_2CO_3, NaBr, and NaCl. Six vials containing diet equilibrated at room temperature and each relative humidity were randomly assigned to the corresponding relative humidity box in each incubator. Four positions were established in each incubator for the boxes to occupy. Every 11 d, the boxes in each incubator were shuffled so that each box spent a total of at least 11 d in each position during the course of the experiment to counteract any temperature variability that may have existed in the incubators. During shuffling, the boxes also were checked to ensure that the salt solutions were still saturated. Environmental conditions in each incubator were monitored using a temperature and relative humidity sensor (HOBO U12, Onset Computer Corporation, Bourne, MA). Live insects in each vial were counted after 46 d by spreading a portion of the contents of a vial on a 9-cm petri dish, which had a coat of Fluon on the walls, and removing motile *L. reticu-*

latus by using a moist brush under a dissecting microscope.

The experiment had two replications over time, and the experimental design was a randomized complete block (RCBD) with subsampling. All statistical procedures were accomplished using Statistical Analysis System software (SAS Institute 2001). PROC MIXED was used for analysis of variance (ANOVA) to determine the effects of temperature and relative humidity on numbers of psocids in vials, which were transformed using the square-root transformation to stabilize variances before analysis. Untransformed means and standard errors are reported to simplify interpretation. We used a least significant difference (LSD) test to determine differences among mean numbers of psocids produced at different temperatures, despite the quantitative independent variable, because we were not able to quantify the relationship using a biologically meaningful equation because of the 35°C data. We did use regression (TableCurve 2D) (Systat Software, Inc. 1996) to describe the relationship between 22.5 and 32.5°C. Selection of an equation to describe the data was based on the magnitude and pattern of residuals, lack-of-fit tests, and whether the curve had a shape that was reasonable for describing the data.

Effects of Marking with Chalk Powder or Methylene Blue. The duration from egg to adult or nymph to adult can be determined easily in parthenogenic *L. reticulatus* because adult psocids possess a characteristic pair of winglets not found in immature stages. However, one cannot easily determine duration of each nymphal stage because the stages are morphologically similar, and the psocids sometimes consume their exuviae. Thus, marking can be used to determine duration of individual nymphal stages. As mentioned in the introduction, there are disadvantages to using methylene blue as a means of marking psocids, so we conducted an experiment to determine whether nontoxic, sidewalk chalk powder (The Crayola Factory, Easton, PA) might be a suitable alternative to methylene blue (Aldrich, Inc., Allentown, PA) for marking psocids to determine number of nymphal stages.

Chalk powder used for marking insects was obtained by breaking a single blue chalk stick into small pieces, and then grinding these into fine powder using a mortar and pestle (Coors Ceramics Co., Golden, CO). An insect was dusted with chalk powder by filling a 35-mm petri dish halfway with chalk powder and transferring the insect onto the powder surface. A dry, fine camel's-hair brush with a small amount of chalk powder was then tapped just above the abdomen of the insect three to four times to ensure there was enough powder stuck on and among hairs on the thorax and abdomen to mark the insect. The insect was then transferred to an experimental arena, and any chalk powder that dropped on the floor of the arena during transfer was completely removed using a moist camel's-hair brush.

To mark insects using methylene blue solution, a fine camel's-hair brush was modified by reducing the number of hairs on it to five. The modified camel's-hair brush was then dipped in 0.5 ml of methylene blue solution in a 35-mm petri dish in such a way as to obtain as little of the methylene blue solution as possible, and this was used to make a small dark blue spot on the abdomen of the insect.

To obtain *L. reticulatus* eggs, 1 g of colored psocid diet and five particles of cracked wheat were placed in each of sixty 35-mm petri dishes, and 30 adult females of unknown age were placed in each petri dish for 24 h to lay eggs. After the 24-h egg laying period, the colored diet in each petri dish was examined for eggs by using a dissecting microscope at 25× magnification. When an egg was found, the diet around it was first loosened using a dissecting pin, after which the egg was transferred into a flat cap of a 1.5-ml centrifuge tube (LabSource Inc., Willowbrook, IL) by using a moist camel's-hair brush. The centrifuge cap was then placed inside a 29- by 94-mm vial (Kimble Glass Inc., Vineland, NJ) cap (25 mm diameter). The walls of both the centrifuge and vial caps were coated with Fluon. The vial cap (with a centrifuge cap inside it) was then placed inside a 35-mm petri dish, and a cracked wheat kernel was placed in each centrifuge cap and vial cap. A single egg transferred from the colored diet was placed on the floor of each of 180 centrifuge caps, and the 35-mm petri dish lids replaced.

Thirty petri dishes containing eggs were then randomly placed in each of six plastic boxes (30 by 23 by 9 cm in height) that had been painted black and contained saturated NaCl solution. This experiment had three treatments, namely, unmarked psocids and those marked using methylene blue or chalk powder. The 30 eggs in each box were randomly assigned to the three treatments, with each treatment receiving 10 eggs. Eggs were examined daily for egg hatch by using a dissecting microscope at 25× magnification. One incubator maintained at 30 ± 1°C was used for the experiment.

Two days after egg hatch, when psocids were strong enough to withstand handling, each first instars (N1) was dusted with blue chalk powder or marked with methylene blue. Each N1 then was transferred from the centrifuge cap into the larger vial cap, and the centrifuge cap plus the cracked wheat particle in it were removed. Nymphs in vial caps were examined daily, using a dissecting microscope at 25× magnification, to monitor development. Absence of blue marking on the abdomen, thorax, or both in treated nymphs indicated a molt had occurred. After each molt, insects in the methylene blue and chalk powder treatments were immediately marked again. In addition, vial caps in all treatments were examined daily for exuviae to determine when a molt had occurred and whether the exuvium had been consumed.

Life history parameters analyzed were duration of incubation, nymphal stages, combined nymphal stages, and combined immature stages, and proportion of nymphs that died in each treatment in each box. The experimental design was a randomized complete block design with subsampling; the experiment had six replications. PROC MIXED was used for ANOVA to

determine the effects of marking. Means were separated using the LSMEANS statement. In the analysis of effect of marking method on mortality of nymphs, PROC GLM was used for ANOVA and means were compared using the LSD method after arcsine square-root transformation to stabilize variances.

Effects of Marking with Fluorescent Powder. The results of the previous marking experiment led us to investigate other alternative marking methods. Fluorescent powder (Day-Glo Color Corp., Cleveland, OH) is much finer than chalk powder and comes in brighter colors, so only very small amounts of it would be required to mark psocids, and this presumably would minimize unintended effects. Methods used were similar to those in the previous marking experiment with a few exceptions. In this experiment, there were only two treatments, namely, insects marked using fluorescent powder and unmarked insects. In addition, 128 eggs were monitored with 16 eggs randomly assigned to each treatment in each of four plastic boxes (30 by 23 by 9 cm in height), and the experiment was replicated four times.

Rocket red fluorescent powder was used to mark insects using a fine camel's-hair brush that was modified by reducing the number of hairs on it to one, and then shortening the length of that hair to 7 mm. The single hair of the modified camel's-hair brush was then gently dipped in a 35-mm petri dish half-full of fluorescent powder in such a way as to obtain as little of the powder as possible, and the powder on the brush was gently rubbed against the abdomen of the psocid to be marked. The psocid was not removed from the vial cap during the marking process. Any fluorescent powder that dropped on the vial cap floor was completely removed using a moist camel's-hair brush.

We also compared the amount of time it takes to mark psocids by using fluorescent powder with that required to mark them using methylene blue. A single person, who was skilled in marking by using both methods, alternately marked 22 N2 psocids by using fluorescent powder or methylene blue on the same occasion, and the time, in seconds, to mark each psocid was determined using a stopwatch.

The experimental design and data analyses were similar to those used in the previous marking experiment. Data on the comparison of times taken to mark psocids by using fluorescent powder or methylene blue were analyzed using PROC ANOVA, and means were compared using the LSD method.

Effects of Temperature on Development. Eggs for this experiment were obtained and placed in centrifuge caps as already described in the experiments to determine the effects of marking. Fifteen centrifuge caps containing eggs were then randomly placed in each of 12 Rubbermaid plastic boxes (30 by 23 by 9 cm in height) that were painted black and contained saturated NaCl solution. Two boxes were placed in each of six incubators set to maintain treatment temperatures of 22.5, 25.0, 27.5, 30.0, 32.5, and 35.0°C. The experiment consisted of three replications over time. We did not test temperatures above 35°C because preliminary experiments had shown that *L. reticulatus* eggs do not hatch at temperatures above 35°C.

The procedures for monitoring egg and nymphal development were similar to those for unmarked individuals in the marking experiment. In this experiment, only exuviae found on the vial caps were used to tell when one developmental stage ended and the next commenced. Collapsed eggs and nymphs mechanically injured during handling were discontinued. Life history parameters measured were the duration of incubation, nymphal stages, combined nymphal stages, and combined immature stages, and proportions of viable eggs and nymphs that developed to the adult stage at each temperature.

In the determination of the effects of temperature on the duration of development of *L. reticulatus*, three data sets were analyzed. The first set of data were for psocids that developed from egg to adult irrespective of the number of exuviae found (all data were used). The second was for those psocids in which four exuviae (the number of expected molts based on the marking study) were found, i.e., psocids that did not consume their exuviae. The last data set was for psocids that consumed their exuviae at least once during their development from egg to adult. For all three data sets, the design used for analysis was a RCBD with subsampling. PROC MIXED was used for ANOVA to determine the effects of temperature on *L. reticulatus* development. The design for data analysis in the comparison between the duration of nymphal development for *L. reticulatus* that consumed their exuviae with those that did not was also an RCBD with subsampling. In the analysis of the proportions of viable eggs and nymphs that developed to the adult stage, the design for analysis was a RCBD. To analyze these proportions, PROC GLM was used for ANOVA after arcsine square-root transformation to stabilize variances. Temperature-dependent development equations for *L. reticulatus* egg, nymphal, combined nymphal, and combined immature stages were developed by regressing developmental times against temperature by using TableCurve 2D (Systat Software, Inc. 1996). A temperature-dependent equation for egg hatch was developed using data from all eggs that hatched, whereas temperature-dependent equations for duration of combined nymphal and combined immature stages were developed using data from all psocids that developed to the adult stage. Finally, using data from all nymphs that developed to adult, we developed a temperature-dependent equation for nymphal survivorship by regressing the proportion of nymphs that survived against temperature by using TableCurve 2D. Temperature-dependent survivorship equations for the individual instars could not be developed because we could determine with certainty timing of molts only for those psocids that survived to the adult stage and for which we found four exuviae. Selection of equations to describe the data was based on the magnitude and pattern of residuals, lack-of-fit tests, and whether the curve had a shape that was reasonable for describing the data.

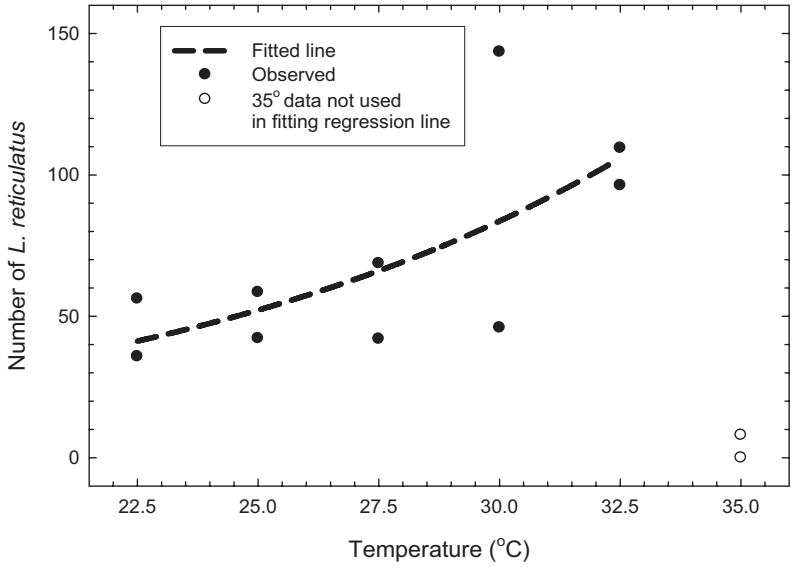

Fig. 1. Population growth of *L. reticulatus* at six constant temperatures and 75% RH. The exponential equation $y = 4.94 \times 10^{(-x/-10.6)}$ (maximum attainable $R^2 = 0.51$; $R^2 = 0.46$; $F = 6.8$; df = 1, 8; $P = 0.03$; lack-of-fit test $P = 0.92$) described the relationship between temperature and *L. reticulatus* population growth between 22.5 and 32.5°C. Open circles indicate data at 35°C that were not used to develop the regression equation, as described in the text.

Results

Effects of Temperature and Relative Humidity on Population Growth. No live *L. reticulatus* were found in treatments maintained at 32, 43, or 55% RH. At 75% RH, psocid populations increased at all temperatures except 35°C; the greatest numbers of psocids were found at 30 and 32.5°C, whereas the lowest number was found at 35°C (ANOVA: $F = 10.4$; df = 5, 5; $P = 0.01$; Fig. 1). Number of psocids produced at 35°C was significantly lower than at all other temperatures (LSD test), and development from 22.5 to 32.5°C was described by an exponential equation.

Effects of Marking with Chalk Powder or Methylene Blue. All *L. reticulatus* marked using either chalk powder or methylene blue had four nymphal stages. ANOVA results indicated that marking with chalk powder did not affect developmental time, but we were concerned that duration of N3, N4, combined nymphal stages, and combined immature stages was considerably shorter than for unmarked psocids. Therefore, we used the LSD test for planned comparisons despite the nonsignificant *F* value (Steel and Torrie 1960). Marking with methylene blue had no effect on the duration of any stage (Table 1). Marking with chalk powder had no effect on the duration of N1 or N2, but it resulted in a reduction in the duration of N3, N4, combined nymphal stages, and combined immature stages compared with unmarked nymphs (Table 1).

Marking with methylene blue increased the mortality of nymphs ($F = 4.08$; df = 2, 10; $P = 0.05$) compared with unmarked nymphs, whereas marking with chalk powder did not affect mortality. The mean ± SE proportions of nymphs that died in the unmarked, methylene blue, and chalk powder treatments were 0.50 ± 0.06, 0.69 ± 0.04, and 0.47 ± 0.06, respectively. Of these proportions, N1 and N2 mortality accounted for 82, 92, and 100%, respectively, of the total mortality.

Effects of Marking with Fluorescent Powder. All *L. reticulatus* marked using fluorescent powder had four nymphal stages. Marking with fluorescent powder had

Table 1. Effect of marking with methylene blue (MB) or chalk powder (CP) on duration of *L. reticulatus* (UM indicates unmarked controls) immature stages

Marking method	*N*	Duration (d ± SE)						
		Egg	N1[a]	N2	N3	N4	Nymphs	Egg + nymphs
UM	18	7.0 ± 0.0a	4.6 ± 0.29a	3.9 ± 0.34a	6.1 ± 0.74a	6.0 ± 0.52a	20.5 ± 1.27a	27.5 ± 1.27a
MB	16	7.0 ± 0.0a	4.6 ± 0.30a	4.7 ± 0.35a	4.7 ± 0.73ab	5.8 ± 0.54ab	19.9 ± 1.29ab	26.9 ± 1.29ab
CP	28	7.0 ± 0.0a	4.7 ± 0.25a	3.9 ± 0.28a	3.6 ± 0.69b	4.5 ± 0.44b	16.7 ± 1.11b	23.7 ± 1.11b

ANOVA results for N1, N2, N3, N4, combined nymphal, and combined immature stages were $F = 0.00$, $P = 0.99$; $F = 1.71$, $P = 0.23$; $F = 3.17$, $P = 0.09$; $F = 3.35$, $P = 0.08$; $F = 3.09$, $P = 0.09$; and $F = 3.09$, $P = 0.09$, respectively; in all cases df = 2, 10. Means within a column followed by the same letter are not significantly different using LSD test.

[a] The four instars of *L. reticulatus* are represented by N1–N4.

Table 2. Effect of marking with fluorescent powder (FP) on duration of *L. reticulatus* (UM indicates unmarked controls) immature stages

Marking method	*N*	Duration (d ± SE)						
		Egg	N1[a]	N2	N3	N4	Nymphs	Egg + nymphs
UM	41	7.2 ± 0.06a	5.2 ± 0.21a	4.2 ± 0.19a	5.0 ± 0.35a	5.6 ± 0.45a	20.0 ± 0.76a	27.1 ± 0.77a
FP	51	7.2 ± 0.05a	5.0 ± 0.18a	3.9 ± 0.18a	4.4 ± 0.22a	4.9 ± 0.28a	18.3 ± 0.56a	25.4 ± 0.57a

ANOVA results for egg, N1, N2, N3, N4, combined nymphal, and combined immature stages were $F = 0.1$, $P = 0.82$; $F = 0.22$, $P = 0.67$; $F = 1.31$, $P = 0.34$; $F = 2.17$, $P = 0.24$; $F = 1.47$, $P = 0.31$; $F = 3.13$, $P = 0.17$; and $F = 3.23$, P = 0.17, respectively; in all cases df = 1, 3. Means within a column followed by the same letter are not significantly different using LSD test.

[a] The four instars of *L. reticulatus* are represented by N1–N4.

no effect on the duration of N1, N2, N3, N4, combined nymphal stages, or combined immature stages (Table 2).

Marking *L. reticulatus* by using fluorescent powder also had no effect on the mortality of nymphs compared with the unmarked nymphs ($F = 0.84$; df = 1, 3; $P = 0.43$). The mean ± SE proportions of nymphs that died in the unmarked and marked treatments were 0.27 ± 0.05 and 0.19 ± 0.04, respectively. Of these proportions, N1 and N2 mortality accounted for 81 and 83%, respectively, of the total mortality.

It took half as much time to mark *L. reticulatus* by using fluorescent powder compared with using methylene blue ($F = 8.0$; df = 1, 20; $P = 0.01$). The mean ± SE times to mark psocids by using fluorescent powder and methylene blue were 26.8 ± 4.3 and 53.5 ± 8.4 s, respectively.

Effects of Temperature on Development. *Eggs.* Based on analysis of data for all eggs that hatched, temperature had a significant effect on the incubation time (Table 3), and a quadratic equation fit the data well (Fig. 2A; Table 4). Incubation time averaged 11.9 d at 22.5°C and declined to 6.4 d at 32.5°C; at 35°C, there was a slight increase in the incubation time. Based on the quadratic model, the predicted optimal incubation temperature is 32.3°C, and development is completed in 6.3 d at this temperature. Temperature had no effect on the viability of eggs ($F = 1.6$; df = 2, 5; $P = 0.26$), which ranged between 80 and 90%.

Nymphs. Based on analysis of data for all individuals that developed to adult, temperature had a significant effect on the combined nymphal developmental period (Table 3), and a quadratic equation fit the data well (Fig. 2B; Table 4). Nymphal developmental time averaged 32.7 d at 22.5°C and declined to 16.6 d at 32.5°C; at 35°C, there was a slight increase in the

Table 3. Duration of immature stages of *L. reticulatus* at six constant temperatures and 75% RH for all eggs that hatched and developed to adult irrespective of whether nymphs consumed exuviae

Temp (°C)	*N*	Duration (d ± SE)		
		Eggs	Nymphs	Eggs + nymphs
22.5	36	11.9 ± 0.38	32.7 ± 1.98	44.6 ± 2.08
25.0	28	9.0 ± 0.38	25.3 ± 2.02	34.3 ± 2.11
27.5	28	7.4 ± 0.38	21.6 ± 2.00	29.1 ± 2.09
30.0	34	7.0 ± 0.38	19.3 ± 1.97	26.3 ± 2.07
32.5	24	6.4 ± 0.38	16.6 ± 2.07	22.9 ± 2.16
35.0	7	6.6 ± 0.45	16.8 ± 2.90	23.4 ± 2.96

ANOVA results for effects of temp on duration of egg and combined nymphal and immature stages were $F = 44.1$, $P < 0.01$; $F = 10.1$, $P < 0.01$; and $F = 18.3$, $P < 0.01$, respectively; in all cases df = 5, 10.

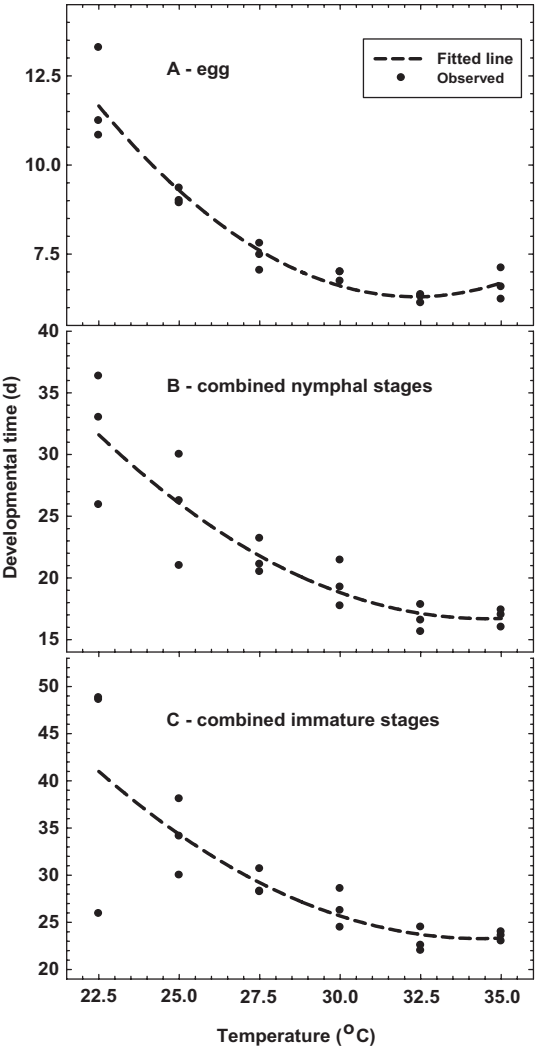

Fig. 2. Development of *L. reticulatus* at six constant temperatures and 75% RH. Graphs show the effects of temperature on (A) egg, (B) combined nymphal stage, and (C) combined immature stage development.

Table 4. Parameters (± SE) for quadratic equations describing nymphal survivorship and the duration of the egg and individual nymphal, combined nymphal, and combined immature stages of *Lepinotus reticulatus* at constant temperatures

Parameter[a]	Max R^2	Adjusted R^2	F	a	b	c
Egg duration	0.93	0.92	100.4	64.2 ± 7.02	−3.58 ± 0.496	0.0553 ± 0.00861
N1 duration*	0.85	0.81	36.2	39.22 ± 9.6801	−2.0245 ± 0.6897	0.0290807 ± 0.012081
N2 duration*	0.86	0.74	25.1	70.555 ± 11.96	−4.4015 ± 0.8518	0.07221 ± 0.01492
N3 duration*	0.69	0.54	11.1	57.64 ± 17.52	−3.354 ± 1.249	0.053089 ± 0.02187
N4 duration*	0.67	0.30	5.0	25.5 ± 27.2	−0.9062 ± 1.94	0.00887 ± 0.0339
Nymphal duration	0.82	0.78	33.6	140 ± 34.0	−7.12 ± 2.40	0.103 ± 0.0417
Egg + nymphal duration	0.65	0.57	13.7	172 ± 63.5	−8.67 ± 4.49	0.126 ± 0.0779
Nymphal survivorship	0.61	0.4	7.7	−2.221 ± 1.566	0.2078 ± 0.1107	−0.004019 ± 0.001921

For parameters with an asterisk, df = 2, 13 and $P < 0.03$. For parameters without an asterisk, df = 2, 15 and $P < 0.01$.

Lack-of-fit P values for the duration of the egg, N1, N2, N3, N4, combined nymphal, and combined immature stages and nymphal survivorship were 0.70, 0.97, 0.23, 0.60, 0.14, 0.96, 0.99, and 0.40, respectively.

[a] N1, N2, N3, and N4 represent the first, second, third, and fourth instars, respectively.

duration of the combined nymphal stages. Based on the quadratic equation, the predicted optimal developmental temperature for nymphs is 34.5°C, and, at this temperature, nymphal development is completed in 16.7 d.

Combined Immature Stages. Based on analysis of data for all individuals that developed to adult, temperature had a significant effect on total developmental time (Table 3), and a quadratic equation fit the data well (Fig. 2C; Table 4). Total developmental time from egg to adult averaged 44.6 d at 22.5°C and declined to 22.9 d at 32.5°C; at 35°C, there was a slight increase in the total developmental time. Based on the quadratic equation, the predicted optimal developmental temperature for immature *L. reticulatus* is 34.4°C, and, at this temperature, development is completed in 23.3 d.

For psocids that did not consume their exuviae, the minimum times required to complete a nymphal stage were recorded at 35°C for N1 (3.8 d) and N4 (4.3 d), whereas the minimum times for N2 (3.5 d) and N3 (4.2 d) were recorded at 27.5 and 32.5°C, respectively (Tables 4 and 5; Fig. 3A–D). The comparison between the duration of nymphal developmental time for *L. reticulatus* that consumed their exuviae with those that did not revealed significant interaction between temperature and exuviae consumption (Fig. 4; $F = 2.9$; df = 5, 116; $P = 0.02$). At 22.5, 25, and 30°C, nymphal developmental time for *L. reticulatus* that consumed their exuviae was shorter than for those that did not consume exuviae (Fig. 4).

A temperature of 35°C had a negative effect on the survivorship of nymphs (Fig. 5). The proportion of nymphs surviving to adults ranged from 0.09 at 35°C to 0.47 at 22.5°C (Fig. 5), and a quadratic equation fit the data well (Table 4).

Discussion

Marking *L. reticulatus* by using methylene blue, chalk powder, and fluorescent powder showed that this psocid species has four instars during its development from egg to adult. Finlayson (1949) found that another species of the genus *Lepinotus*, *L. patruelis*, has five instars. Although marking psocids by using methylene blue is the method that has been used in past studies (Mockford 1957, Leong and Ho 1995), our work showed that the use of this method takes longer and leads to increased insect mortality. Using chalk powder to mark psocids does not increase insect mortality, but it results in a reduction in developmental time for N3, N4, combined nymphal stages, and combined immature stages. Studies conducted using the silkworm, *Bombyx mori* L., have shown that calcium carbonate, an ingredient in the chalk powder that we used, stimulates larval appetite (Ikejima et al. 2004, Tsuneyama et al. 2005). Given that psocids have a tendency to consume their exuviae, consuming exuviae containing chalk powder, and thus calcium carbonate, may increase the appetite of nymphs thereby increasing their nutrient intake and shortening devel-

Table 5. Duration of immature stages of *L. reticulatus* at six constant temperatures and 75% RH for insects in which four exuviae that corresponded to the number of expected molts were found

Temp (°C)	n	Duration (d ± SE)						
		Egg	N1[a]	N2	N3	N4	Nymphs	Eggs + nymphs
22.5	19	12.0 ± 0.38	8.7 ± 0.54	8.7 ± 0.59	9.1 ± 0.76	9.8 ± 1.06	36.2 ± 1.64	48.1 ± 1.71
25	12	8.9 ± 0.45	6.5 ± 0.63	6.1 ± 0.74	7.0 ± 0.90	9.2 ± 1.28	28.8 ± 1.95	37.9 ± 2.00
27.5	9	7.8 ± 0.41	5.7 ± 0.68	3.5 ± 0.84	5.4 ± 0.94	6.0 ± 1.41	20.8 ± 2.15	28.3 ± 2.19
30	11	7.0 ± 0.39	4.5 ± 0.63	3.8 ± 0.76	5.5 ± 0.86	8.2 ± 1.29	22.1 ± 1.96	29.1 ± 2.00
32.5	9	6.3 ± 0.41	4.1 ± 0.68	4.4 ± 0.84	4.2 ± 0.93	4.7 ± 1.41	17.3 ± 2.13	23.6 ± 2.18
35	2	7.5 ± 0.64	3.8 ± 1.28	4.5 ± 1.76	5.5 ± 1.78	4.3 ± 2.83	17.9 ± 4.23	25.2 ± 4.23

ANOVA results for egg, N1, N2, N3, N4, combined nymphal, and combined immature stages were $F = 26.8$, $P < 0.01$; $F = 13.2$, $P = 0.01$; $F = 8.1$, $P < 0.01$; $F = 4.2$, $P = 0.04$; $F = 3.0$, $P = 0.08$; $F = 18.4$, $P < 0.01$; and $F = 31.5$, $P < 0.01$, respectively; in all cases df = 5, 10.

The four instars of *L. reticulatus* are represented by N1–N4.

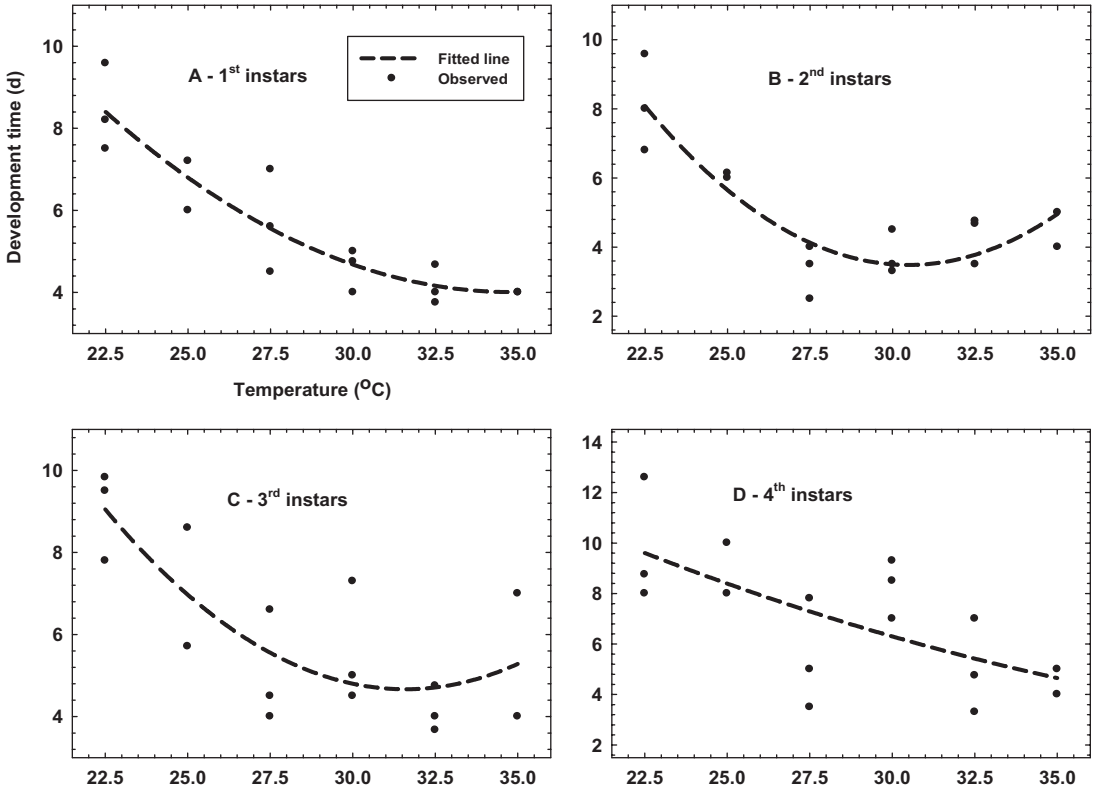

Fig. 3. Development of *L. reticulatus* at six constant temperatures and 75% RH. Graphs show the effects of temperature on the development of (A) first, (B) second, (C) third, and (D) fourth instars.

opmental time. Other calcium compounds such as calcium phosphate monobasic and calcium lactate have been used as components of insect artificial diets (Fraenkel and Blewett 1943), and it is possible that their main role also could be stimulating insect appetites. Therefore, marking psocids by using chalk powder is not a suitable replacement for methylene blue.

We found that marking psocids by using fluorescent powder is faster (takes half as much time as using methylene blue) and does not affect insect mortality or developmental time. Therefore, we recommend that fluorescent powder be used as a replacement for methylene blue as a means of marking psocids during studies on their development.

In parthenogenic psocids where adults have unique characteristics not found in immatures and where the number of instars is known, it is not necessary to mark psocids during life cycle studies. During our study to determine the effects of temperature on the development of *L. reticulatus*, we already knew the number of nymphal instars present because of results from our marking study. Therefore, we did not mark psocids. The disadvantage of not marking psocids was that 61% of the psocids that developed to the adult stage consumed their exuviae at least once. When psocids are not marked, data from insects that eat their cast skins cannot be used during analysis to determine the duration of the various instars, thereby weakening the power of the experiment to define the values of these parameters.

L. reticulatus will survive and multiply predictably at temperatures between 22.5 and 32.5°C and 75% RH; however, *L. reticulatus* will not survive at RH of 32, 43, and 55%. According to Devine (1982), psocids maintain body water levels by absorbing atmospheric water vapor when RH is 60% or above; however, below this level, more water is lost than gained resulting in dehydration and death. We also found that a temperature of 35°C has a detrimental effect on the survival and reproduction of *L. reticulatus.* Although 33–47% of nymphs survived to adulthood within the 22.5–32.5°C temperature range, only 9% of the nymphs survived to adults at 35°C. This may partly explain the retarded population growth of *L. reticulatus* at 35°C. During development, we found that 80–100% of the total mortality was due to N1 and N2 mortality. Our results are supported by the work of Rees and Walker (1990), who also found that none of the three psocid species they studied (*Liposcelis bostrychophila* Badonnel, *L. entomophila*, and *L. paeta* Pearman) was able to survive at relative humidities below 60%. According to their study, only *L. paeta* was able to survive at temperatures higher than 36°C.

Our work shows that the predicted optimal temperatures for egg, combined nymphal development, and combined immature development are 32.3, 34.5,

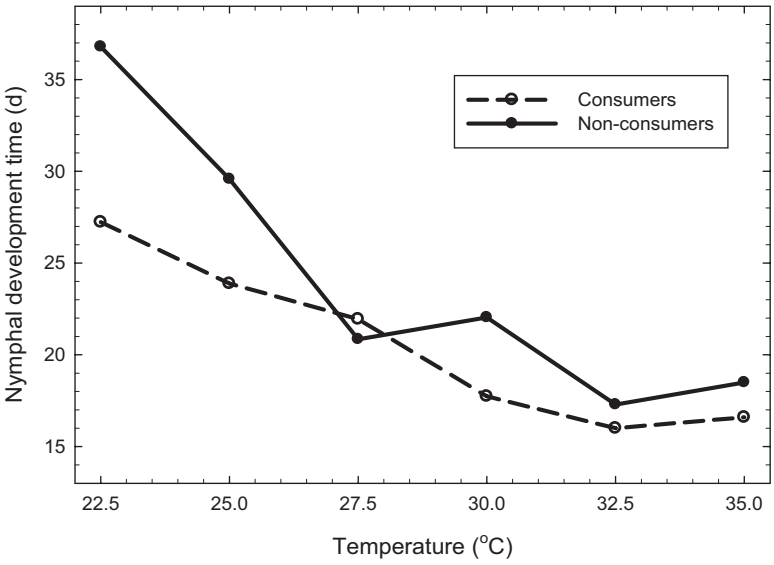

Fig. 4. Duration (days ± SE) of nymphal development in *L. reticulatus* that consumed their exuviae and those that did not at six constant temperatures and 75% RH. There were significant differences between developmental times for exuviae consumers and nonconsumers at 22.5, 25, and 30°C ($t = -4.66$, $P < 0.01$; $t = -2.76$, $P = 0.07$; and $t = -2.44$, $P = 0.02$, respectively), but not at 27.5, 32.5 and 35°C ($t = 0.55$, $P = 0.58$; $t = -0.80$, $P = 0.42$; and $t = -0.47$, $P = 0.64$, respectively).

and 34.4°C, respectively. At these temperatures, development is completed in 6.3, 16.7, and 23.3 d, respectively. The trend is for duration of these stages to decrease with temperature up to 32.5°C after which developmental time starts to increase slightly. A similar trend was found for a different psocid species, *L. bostrychophila* (Wang et al. 2000). The temperature-dependent equations for development of *L. reticulatus* eggs, combined nymphal, and combined immature stages and nymphal survivorship can provide valuable information that can help elucidate the population dynamics of a particular species (Summers et al. 1984), despite that insects do not live in environments with constant temperatures, and they can be used to develop effective management strategies for this psocid.

At 22.5, 25, and 30°C, we found the duration of the combined nymphal stage for psocids that did not consume their exuviae to be 8, 5, and 4 d, respectively, longer than in psocids that did. The proportion change in nymphal developmental time, at these three temperatures, was higher at 22.5°C (25 versus 19%), and we found the same pattern for total developmental

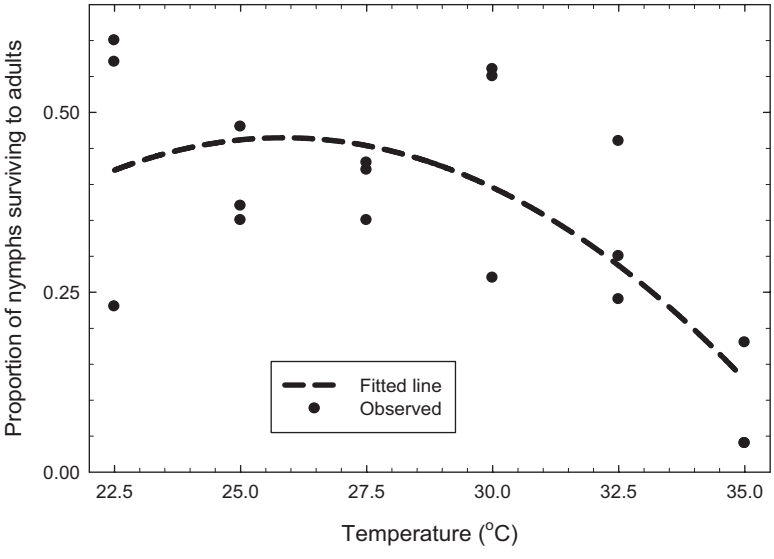

Fig. 5. Survivorship of *L. reticulatus* nymphs at six constant temperatures and 75% RH.

time. These differences were not as dramatic at temperatures of 27.5, 32.5, and 35°C; however, the general trend was that development was slower for psocids that did not consume exuviae. The possible reason for this could lie in the nutritional status of exuviae. Lipids and nitrogenous compounds (protein and chitin) can account for as much as 4.4% (Nelson and Sukkestad 1975) and 87% (Mira 2000), respectively, of the total weight of insect exuviae. Therefore, exuviae would be beneficial to psocids at all temperatures. We found the effects of consuming exuviae to be more pronounced at low temperatures. It is likely that at low temperatures, feeding and digestion may be slowed down; therefore, at these low temperatures, feeding on a concentrated source of protein and lipid may be more advantageous and could explain the more pronounced effect observed. At higher temperatures, where insects are more active, most of the nutritional requirements could be met by feeding on wheat that the insects were reared on; hence, the benefit of consuming exuviae would not be so pronounced.

Our work has shown that *L. reticulatus* has six developmental stages, namely, egg, four instars, and the adult stage. In addition, we have presented a new, user-friendly method for marking psocids during life history studies. Finally, we have developed temperature-dependent developmental equations plus a nymphal survivorship equation which can be used to understand better *L. reticulatus* population dynamics and to develop effective management strategies.

Acknowledgments

We thank Ann Redmon and Ngunza Kisangani for technical support; we also thank Maria Otilia Carvalho, Edward Mockford, and Manoj Nayak for reviewing an earlier draft of this manuscript. In addition, we would like to extend our appreciation to Edward Mockford who confirmed the identity of *L. reticulatus*.

References Cited

Devine, T. L. 1982. The dynamics of body water in the booklouse *Liposcelis bostrychophilus* (Badonnel). J. Exp. Zool. 222: 335–347.

Finlayson, L. H. 1949. The life history and anatomy of *Lepinotus patruelis* Pearman. Proc. Zool. Soc. Lond. 119: 301–323.

Fraenkel, G., and M. Blewett. 1943. The basic food requirements of several insects. J. Exp. Biol. 20: 28–34.

Greenspan, L. 1977. Humidity fixed points of binary saturated aqueous solutions. J. Res. Natl. Bur. Stand. A 81: 89–96.

Ikejima, S., I. Tsuneyama, N. Iida, Y. Tsurui, O. Ninagi, and Y. Tanaka. 2004. Effects of calcium carbonate and saccharides on the feeding behavior of newly hatched larvae of the silkworm, *Bombyx mori* L. Jpn. J. Appl. Entomol. Zool. 48: 331–336.

Leong, E.C.W., and S. H. Ho. 1995. Life cycle of *Liposcelis entomophila* (Psocoptera: Liposcelididae) and a culturing regime for liposcelids. Bull. Entomol. Res. 85: 501–506.

Lienhard, C., and C. N. Smithers. 2002. Pscoptera (Insecta) world catalogue and bibliography. Instrumenta Biodiversitatis V, Museum d'histoire naturelle, Geneva, Switzerland.

Mashaya, N. 1999. Population dynamics of *Liposcelis entomophila* (Enderlein) (Pscocoptera: Liposcelididae) in farm tobacco processing buildings. J. Stored Prod. Res. 35: 355–368.

Mashaya, N. 2001. Influence of ambient temperature and relative humidity on changes in numbers of a natural population of *Liposcelis entomophila* (Enderlein) (Pscocoptera: Liposcelididae). J. Stored Prod. Res. 37: 1–12.

Mira, A. 2000. Exuviae eating: a nitrogen meal? J. Insect Physiol. 46: 605–610.

Mockford, E. L. 1957. Life history studies of some Florida insects of genus *Archipsocus* (Psocoptera). Bull. Fla. Mus. Nat. Hist. Biol. Sci. 1: 253–275.

Mockford, E. L. 1993. North American Psocoptera. The Sandhill Crane Press, Inc., Gainesville, FL.

Nayak, M. K. 2006. Psocid and mite pests of stored commodities: small but formidable enemies, pp. 1061–1073. *In* I. Lorini, B. Bacaltchuk, H. Beckel, D. Deckers, E. Sundfeld, J. P. dos Santos, J. D. Biagi, J. C. Celaro, L. R. D'A. Faroni, L de O. F. Bortolini, et al. [eds.], Proceedings of the 9th International Working Conference on Stored Product Protection, 15–18 October 2006, São Paulo, Brazil. Brazilian Post-Harvest Association–ABRAPOS, Passo Fundo, RS, Brazil.

Nayak, M. K., P. J. Collins, and S. R. Reid. 1998. Efficacy of grain protectants and phosphine against *Liposcelis bostrychophila, L. entomophila,* and *L. paeta* (Psocoptera: Liposcelidae). J. Econ. Entomol. 91: 1208–1212.

Nayak, M. K., P. J. Collins, and H. Pavic. 2002a. Resistance to phosphine in psocids: challenges ahead!, pp. 113–118. *In* E. J. Wright, H. J. Banks, and E. Highley (Eds), Proceedings of the 2nd Australian Postharvest Technical Conference, 1–4 August 2000, Adelaide, Australia. Stored Grain Research Laboratory, Canberra, Australia.

Nayak, M. K., P. J. Collins, and H. Pavic. 2002b. Long-term effectiveness of grain protectants and structural treatments against *Liposcelis decolor* (Pearman) (Psocoptera: Liposcelididae), a pest of stored products. Pest Manag. Sci. 58: 1223–1228.

Nayak, M. K., P. J. Collins, H. Pavic, and R. A. Kopittke. 2003. Inhibition of egg development by phosphine in the cosmopolitan pest of stored products *Liposcelis bostrychophila* (Psocoptera: Liposcelididae). Pest Manag. Sci. 59: 1191–1196.

Nelson, D. R., and D. R. Sukkestad. 1975. Normal and branched alkanes from cast skins of the grasshopper *Schistocerca vaga* (Scudder). J. Lipid Res. 16: 12–18.

Rees, D. P., and A. J. Walker. 1990. The effect of temperature and relative humidity on population growth of three *Liposcelis* species infesting stored products in tropical countries. Bull. Entomol. Res. 80: 353–358.

SAS Institute. 2001. The SAS system for Windows, version 8. SAS Institute, Cary, NC.

Sinha, R. N. 1988. Population dynamics of Psocoptera in farm-stored grain and oilseed. Can. J. Zool. 66: 2618–2627.

Steel, R.G.D., and J. H. Torrie. 1960. Principles and procedures of statistics. McGraw-Hill, New York.

Summers, C. G., R. L. Coviello, and A. P. Gutierrez. 1984. Influence of constant temperature on the development and reproduction of *Acyrthosiphon kondoi* (Homoptera: Aphididae). Environ. Entomol. 13: 236–242.

Systat Software, Inc. 1996. TableCurve 2D, version 4.0. Systat Software, Inc., Point Richmond, CA.

Throne, J. E., G. P. Opit, and P. W. Flinn. 2006. Seasonal distribution of psocids in stored wheat, pp. 1095–1103. *In* I. Lorini, B. Bacaltchuk, H. Beckel, D. Deckers, E. Sundfeld,

J. P. dos Santos, J. D. Biagi, J. C. Celaro, L. R. D'A. Faroni, L de O. F. Bortolini, et al. [eds.], Proceedings of the 9th International Working Conference on Stored Product Protection, 15–18 October 2006, São Paulo, Brazil. Brazilian Post-Harvest Association–ABRAPOS, Passo Fundo, RS, Brazil.

Tsuneyama, I., S. Ikejima, N. Iida, Y. Tsurui, Y. Tanaka, O. Ninagi, and T. Oshiki. 2005. Combined effect of calcium carbonate and sugar on the feeding behavior of the newly hatched larvae of the silkworm, *Bombyx mori.* J. Seric. Sci. Jpn. 74: 9–14.

Wang, J. J., Z. Zhao, and L. Li. 1998. Studies on bionomics of *Liposcelis entomophila* (Psocoptera: Liposcelididae) infesting stored products. Entomol. Sin. 5: 149–158.

Wang, J. J., J. H. Tsai, Z. M. Zhao, and L. S. Li. 2000. Development and reproduction of the psocid *Liposcelis bostrychophila* (Psocoptera: Liposcelididae) as a function of temperature. Ann. Entomol. Soc. Am. 93: 261–270.

Received 15 March 2007; accepted 27 September 2007.